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TRACKING CENTRAL HYPOVOLEMIA WITH ECG IN HUMANS: CAUTIONS FOR THE USE OF HEART PERIOD VARIABILITY IN PATIENT MONITORING

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ABSTRACT—Heart period variability (HPV) metrics have been suggested for use in medical monitoring of trauma patients. This study sought to ascertain the use of various HPV metrics in tracking central blood volume during simulated hemorrhage in individual humans. One hundred one healthy nonsmoking volunteers (58 men, 43 women) were instrumented for continuous measurement of electrocardiogram and beat-by-beat finger arterial blood pressure. Stroke volume (SV) was estimated from the arterial pulse wave and used to reflect central blood volume. Progressive lower body negative pressure (LBNP) was applied in 5-min stages until the onset of impending hemodynamic decompensation (systolic blood pressure <70 mmHg and/or presyncopal symptoms). HPV was assessed with analysis of R-to-R intervals using both linear (time and frequency domains) and nonlinear (e.g., complexity, fractality) methods. Application of increasing LBNP caused progressive reductions of SV, whereas arterial pressures changed only minimally and late. Group LBNP stage means for each HPV metric changed progressively and were strongly correlated with the mean decrease in SV ($|r| \geq 0.87$). To ascertain the utility of the HPV metrics to track individual responses to central hypovolemia, the difference scores for each HPV metric were correlated at each successive LBNP level, with percentage change in SV at the subject level. This cross-correlation of difference scores revealed that none of the HPV metrics showed strong and consistent correlations ($|r| \leq 0.49$) with percentage change in SV across successive LBNP levels. Although aggregate group mean values for HPV metrics are well correlated with SV changes during central hypovolemia, these metrics are less reliable when tracking individual reductions in central volume during LBNP. HPV metrics, therefore, may not be useful in monitoring hemorrhagic injuries in individual patients.

KEYWORDS—Heart rate variability, hemorrhage, trauma, lower body negative pressure

INTRODUCTION

Hemorrhage remains a leading cause of death after traumatic injury in both the military and civilian settings (1, 2). Currently, prehospital medics must make triage decisions based on standard vital signs such as noninvasive blood pressures, heart rate, arterial oxygen saturation, and basic neurological assessments (e.g., via Glasgow Coma Scale scores). Although abnormalities in these vital signs offer a clear indication for intervention or evacuation, they usually occur late in the time course of progressive blood loss because of compensatory physiological mechanisms that act to maintain blood pressure and oxygen saturation at normal levels until the point of decompensation (3, 4). Because of this, valuable time might be lost before medical intervention (e.g., resuscitation) is deemed necessary, reducing the likelihood of a positive outcome (4). Identification of noninvasive measures that reflect early autonomic and hemodynamic compensatory responses could therefore facilitate early diagnosis and precipitate resuscitative efforts before the onset of hemodynamic decompensation.

Although heart rate itself is neither sensitive nor specific in determining the presence of hypotension or severe injury in trauma patients (5–7), indices of heart period variability (HPV) have been proposed as potentially useful measures to assist triage decisions in trauma patients (3, 8–11). These data from prehospital trauma patients are consistent with reported observations that both linear and nonlinear indices of HPV decrease with progressive blood loss in animal models of hemorrhagic shock (12–15) and human models of central hypovolemia (3, 16–18). This is not unexpected because linear measures, in particular, reflect modulations in autonomic control of the cardiovascular system (19). In fact, recent observations suggest that a progressive reduction in central blood volume in humans elicits reductions in several HPV indices that are strongly correlated with increases in directly measured sympathetic nerve activity (20). It is unknown, however, whether changes in these indices are directly associated with changes in central blood volume during hypovolemia.

The suggestion that HPV parameters could be used in monitoring trauma patients has been based primarily on determining average values of these metrics, either during experimental hypovolemia models or in groups of trauma patients. Although an important initial step in elucidating physiological processes, calculation of group means may mask individual variability. Before asserting that these metrics be used to monitor individual trauma patients during suspected hemorrhagic events, it is necessary to determine whether they will be indicative of the level of central hypovolemia on an individual patient basis. In the present study, we assessed group and individual HPV responses in human

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subjects exposed to an experimentally induced progressive reduction in central blood volume (simulated progressive hemorrhage). We hypothesized that group means of HPV metrics would be strongly correlated with corresponding mean progressive reductions in central blood volume (as measured by stroke volume [SV]), but that high interindividual variability in these responses would limit the predictive utility of these parameters for monitoring individuals with hemorrhagic injuries.

MATERIALS AND METHODS

Subjects

One hundred one (58 men, 43 women) healthy nonsmoking subjects volunteered to participate in this study (mean age [± SD], 28 ± 8 years; height, 173 ± 11 cm; weight, 76 ± 15 kg). Before inclusion, all subjects completed a medical history and physical examination by a physician to ensure that they had no previous or current medical conditions that might preclude their participation. All female subjects underwent a urine pregnancy test within 24 h of experimentation and were excluded if pregnant. All subjects maintained their normal sleep patterns, refrained from exercise, and abstained from caffeine and other autonomic stimulants including prescription or nonprescription drugs at least 24 h before the experimental procedure. Subjects received a verbal briefing and written descriptions of all procedures and risks associated with the study and were made familiar with the laboratory, the protocol, and procedures. Subjects were encouraged to ask questions to the investigators and then signed an informed consent form that had been approved by the institutional review board for the protection of human subjects in research from Brooke Army Medical Center and The US Army Institute of Surgical Research, Fort Sam Houston.

Experimental protocol

Subjects were instrumented with a standard 4-lead electrocardiogram (ECG) to record cardiac electrical potentials, and an inflatable finger cuff to record beat-by-beat finger arterial pressure and SV (Finometer Blood Pressure Monitor; TNO-TPD Biomedical Instrumentation, Amsterdam, The Netherlands). The Finometer blood pressure cuff was placed on the middle finger of the left hand, which in turn was laid at heart level. Central hypovolemia was induced by application of LBNP to simulate, as closely as possible in healthy human volunteers, the hemodynamic challenges associated with severe hemorrhage (21–23). Acute cardiovascular and sympathoexcitatory effects of LBNP have been shown to be similar to those produced by hemorrhage (22, 23), and there is a high level of reproducibility within subjects in LBNP tolerance time (24). Subjects were positioned supine within an airtight chamber that was sealed at the level of the iliac crest using a neoprene skirt. Because injured patients do not breathe at a fixed rate, we did not attempt to control breathing frequency. Each subject underwent exposure to a progressive LBNP protocol that consisted of a 5-min control period (baseline), followed by 5 min of chamber decompression at −15, −30, −45, and −60 mmHg, then additional increments of −10 mmHg every 5 min until the onset of hemodynamic decompensation, followed by a 10-min recovery period. Hemodynamic decompensation was identified in real time by the attending investigator by a precipitous fall in systolic pressure greater than 15 mmHg, progressive diminution of systolic pressure less than 70 mmHg, and/or voluntary subject termination caused by discomfort from symptoms such as gray-out (loss of color vision), tunnel vision, sweating, nausea, or dizziness.

Data analysis

All data were sampled at 500 Hz, digitized, and recorded to data acquisition software (WINDAQ; Dataq Instruments, Akron, Ohio). Waveform data were then imported into commercially available data analysis software (WinCPRS; Absolute Aliens, Turku, Finland). The last 3 min of data from each LBNP stage were extracted for analysis. Beat-to-beat SV was estimated

TABLE 1. **Definition of each HPV metric of interest**

Metric	Abbreviation	Unit	Description
Time domain			
RRI SD	RRISD	ms	The SD of RRIs within a specified time
RRI root mean squared SD	RMSSD	ms	Root mean square difference among successive RRIs
pNN50	pNN50	%	The percentage of adjacent RRIs that varied by at least 50 ms
Poincaré plot descriptor-SD-1	SD-1	—	The SD measuring the dispersion of points across the line of identity of a Poincare plot (44–46)
Poincaré plot descriptor-SD-2	SD-2	—	The SD measuring the dispersion of points along the line of identity of a Poincare plot (44–46)
Complex demodulation LF	CDM LF	—	The amplitude of low-frequency oscillations in the RRI signal (47)
Complex demodulation HF	CDM HF	—	The amplitude of high-frequency oscillations in the RRI signal (47)
Frequency domain			
RRI low-frequency power	RRI LF	ms^2	Power spectral density of the low-frequency oscillations (0.04–0.15 Hz) of the RRI (48)
RRI high-frequency power	RRI HF	ms^2	Power spectral density of the high-frequency oscillations (0.15–0.4 Hz) of the RRI (48)
Complexity			
Sample entropy	SampEn	—	A measure of the regularity of the RRI signal, similar to ApEn but less dependant on record length (49)
Lempel-Ziv entropy	LZEn	—	A measure of the regularity or randomness of the RRI signal (40)
Fractal dimensions by curve length	FD-L	—	A measure of the fractal nature (self similarity) of the RRI signal. High FD-L indicates a more complex signal (40, 50, 51)
Fractal dimensions by dispersion analysis	FD-DA	—	A measure of the fractal nature (self similarity) of the RRI signal. High FD-DA indicates a more complex signal (40, 50, 51)
Symbol dynamics entropy	SymDyn	—	A measure of the probability of particular patterns or sequences occurring within an RRI signal (40, 52)
Stationarity	StatAv	—	The stability of the RRI signal; the tendency of the mean to vary with time. Smaller values denote greater stationarity of the signal (53)
Normalized symbol dynamics entropy	DisnEn	Bits/word (BPW)	A method of normalizing symbol dynamics entropy (40)
Detrended fluctuations analysis	DFA	—	Determines long (DFA long) and short (DFA short) range correlations in the RRI signal (54)

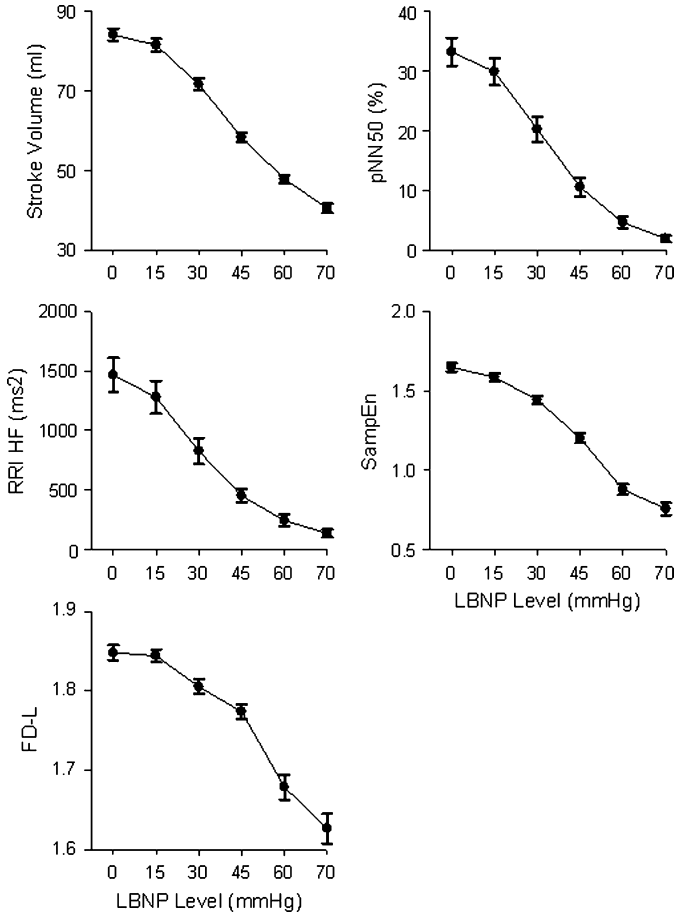

FIG. 1. **Stroke volume and selected HPV metrics (pNN50, RRI HF, SampEn, FD-L) during LBNP**. Data are means ± SE; n = 101.

noninvasively as an indicator of central blood volume from changes in pulse waveforms measured by the Finometer. Stroke volume estimation via application of infrared photoplethysmography (e.g., Finometer) is based on computed aortic flow pulsations from arterial pressure waveforms by simulating a nonlinear time-varying three-element model (aortic characteristic impedance, arterial compliance, and systemic vascular resistance) of aortic input impedance (25). Blood pressure measurement and SV estimation using the Finometer have been demonstrated to be accurate, reliable, and reproducible for physiological studies (reviewed in (26, 27)).

All ECG signals were manually scanned for noise, ectopy, or aberrant beats. If a single ectopic beat was detected within the 3-min analysis period, it was linearly interpolated so that the data could be used for analysis (occurred in five subjects); if there was more than one ectopic beat at a given LBNP level, the data for that level were not analyzed, but the data from all remaining levels were included (occurred in seven subjects). Individual R-waves generated from the ECG were marked at their occurrence in time and used for subsequent identification of systolic and diastolic pressures generated from the Finometer. HPV was assessed in the time and frequency domains and also by using nonlinear metrics to determine complexity features of the ECG (see Table 1 for a list and definitions of metrics assessed). For representation of HPV in the frequency domain, R-R intervals (RRIs) were replotted using linear interpolation and resampled at 5 Hz. Data were then passed through a low-pass impulse response filter with a cutoff frequency of 0.5 Hz. Data sets were submitted to a Fourier transform with a Hanning window. In our analysis, we used only those metrics previously determined to require approximately 200 heartbeats for valid measurement (28), a condition met even during the baseline 3-min measurement period (194 ± 30 beats).

Statistical analysis

Analysis was accomplished using commercially available software (SigmaStat; Systat Software, Richmond, Calif; and SAS and JMP; SAS Institute, Cary, NC). Before analysis, the overall distributional characteristics of each HPV metric were examined and when appropriate, normalizing transformations were applied.

To describe overall changes in the HPV metrics and SV over LBNP stages, the initial statistical analysis consisted primarily of the analysis of LBNP stage means. One-way repeated-measures ANOVA corrected for the autocorrelation structure of the data (i.e., mixed model) was used for testing statistical differences across LBNP levels for each dependent variable (i.e., HPV metrics and SV). Amalgamated Pearson correlation coefficients (i.e., correlations on mean values) were calculated as a general summary statistic for describing the concurrency between LBNP means for the metrics with percentage change in SV.

To ascertain whether HPV metrics would track individual responses to central hypovolemia (i.e., percentage change in SV) during LBNP, difference scores were calculated between successive LBNP levels for each metric. The difference scores between successive LBNP levels were then correlated with percentage changes in SV at each LBNP level across subjects. These dynamic correlations reflect the degree of concordance between percentage changes in SV and changes in the metrics as they occurred between each of the six successive LBNP levels. High correlations reflect high concordance, whereas low correlations indicate that changes in the variable of interest are not primarily associated with changes in percentage SV. Unlike the analysis of means across LBNP levels, the consistency of the correlations across LBNP stages indicates concordance at more of an individual level (i.e., unit change per unit change) across the complete LBNP protocol. Similarly, only the sign of the change scores (i.e., +/−) were analyzed separately by cross tabulating (i.e., 2 × 2 contingency tables) each HPV metric with percentage change in SV at each successive level of LBNP. This dichotomizing of the change scores (i.e., only retaining the signs of the change scores) allowed for a more epidemiological interpretation of the results through the calculation of the false-negative rate (i.e., percentage of subjects changing opposite to the predominantly decreasing SV) for each metric (i.e., test variable) against percentage change in SV (i.e., independent variable).

RESULTS

Figure 1 depicts group mean responses in SV, as well as four examples of HPV metrics to application of progressive LBNP. Stroke volume decreased incrementally, as did both linear (e.g., the percentage of adjacent RRIs that varied by at least 50 ms [pNN50] and RRI high-frequency power [RRI HF]) and nonlinear (e.g., sample entropy [SampEn] and fractal dimensions by curve length [FD-L]) metrics. Statistically, all of the mean changes shown in Figure 1 across LBNP were very large, with corresponding univariate F statistics in excess of 50. Differences in LBNP means were statistically significant ($P < 0.001$) even under the most restrictive and conservative repeated measures models. With the exception of complex demodulation in the low-frequency range (CDM LF), large

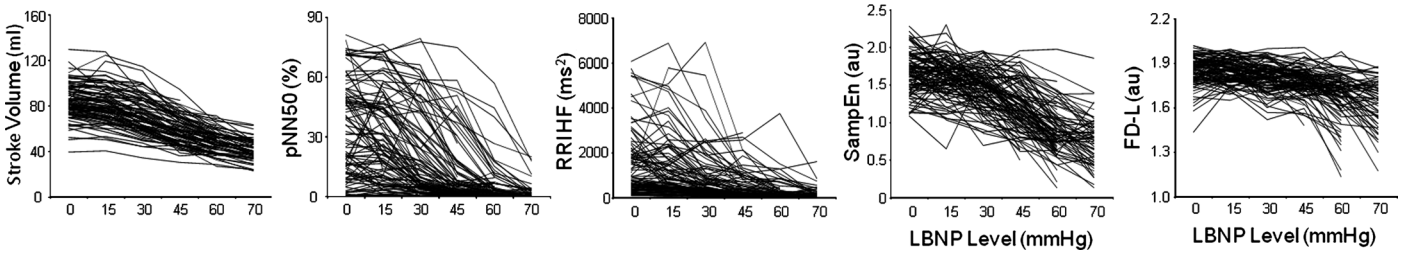

FIG. 2. **Individual subject trajectories for SV and selected HPV metrics (pNN50, RRI HF, SampEn, FD-DA) during LBNP.**

TABLE 2. **Table values are correlations of change scores at successive LBNP stages with percentage change in SV (i.e., lag 1 differences cross correlations)**

	LBNP stage				
Metric	0 to −15 (n = 101)	−15 to −30 (n = 101)	−30 to −45 (n = 101)	−45 to −60 (n = 82)	−60 to −70 (n = 49)
RRISD	0.02	0.00	0.11	0.10	0.22
$RMSSD_{ln}$	0.18	**0.25**	**0.35**	−0.02	**0.49**
$pNN50_{sqrt}$	0.13	**0.22**	**0.41**	**0.27**	0.07
$SD1_{sqrt}$	0.14	0.08	**0.34**	**0.25**	**0.41**
SD2	−0.02	−0.02	0.08	0.11	**0.34**
SD2/SD1*	−**0.29**	−**0.24**	−0.19	0.08	−**0.28**
CDM LF	−0.02	−0.13	0.10	0.04	**0.44**
CDM HF	0.19	0.09	**0.24**	**0.30**	**0.35**
$RRI\ LF_{ln}$	−0.08	−0.19	0.03	−0.03	**0.43**
$RRI\ HF_{ln}$	0.12	0.18	0.17	0.15	**0.49**
SampEn	0.04	**0.25**	**0.24**	0.19	0.03
LZEn	0.09	**0.24**	0.01	0.07	0.21
FD-L	0.04	0.05	0.13	0.01	**0.34**
FD-DA	0.14	−0.09	0.04	**0.26**	0.27
SymDyn	0.09	0.19	0.17	0.12	0.15
StatAv*	−0.13	0.00	−**0.26**	−0.20	−**0.31**
BPW	0.08	0.18	0.17	0.15	0.15
DFA long*	−0.09	0.05	−0.19	−0.20	−**0.30**

Coefficients in bold font reflect associated values of $P < 0.05$.
*Inversely correlated with SV and, therefore, the expected signs are negative.

differences caused by the application of LBNP were observed for all 19 HPV measurements regardless of the parameters used to define the intercorrelation structure placed on the statistical model. As expected, because of the smoothing effect of averaging, aggregate correlations between mean values of the metrics at each LBNP level and mean values of SV at each LBNP level were high for all metrics ($|r| \geq 0.87$).

Figure 2 illustrates individual trajectories of the same HPV metrics for which group means are presented in Figure 1. Despite the progressive decreases in these variables demonstrated

TABLE 3. **Table values are false-negative rates (%) produced when each variable is used to track the % change in SV at each LBNP stage**

	LBNP Stage				
Metric	0 to −15 (n = 101)	−15 to −30 (n = 101)	−30 to −45 (n = 101)	−45 to −60 (n = 82)	−60 to −70 (n = 49)
RRISD	60	34	30	30	16
$RMSSD_{ln}$	35	**12**	**10**	**6**	**12**
$pNN50_{sqrt}$	38	16	**13**	25	31
$SD1_{sqrt}$	38	**4**	**1**	**0**	**0**
SD2	65	37	33	30	18
SD2/SD1	24	**15**	**9**	**11**	20
CDM LF	51	49	49	29	18
CDM HF	39	17	**14**	**9**	16
$RRI\ LF_{ln}$	47	51	45	35	22
$RRI\ HF_{ln}$	36	17	18	**15**	16
SampEn	40	25	17	**5**	16
LZEn	42	35	24	**14**	20
FD-L	39	35	41	24	27
FD-DA	43	39	50	34	35
SymDyn	39	25	19	**9**	**12**
StatAv	38	40	39	30	33
BPW	39	24	18	**8**	**12**
DFA long	43	41	42	34	31

False negative rates less than or equal to 15% are in bold font.

by the average LBNP group values, individuals demonstrated a wide range of responses at each level of LBNP. Although it is evident that some subjects demonstrated progressive declines in the representative HPV metrics depicted, it is also obvious that the responses of these metrics in other individuals was highly variable and unpredictable throughout the LBNP. Table 2 presents results of the correlation analysis between percentage change scores of SV and change scores of each metric at each LBNP level. No metric showed consistent correlations across all five successive LBNP intervals; pNN50, Poincaré plot descriptor SD-1, and complex demodulation in the high-frequency range (CDM HF) showed consistent correlations in three of five consecutive LBNP intervals. Frequency-domain variables (RRI HF and RRI low-frequency power [RRI LF]) were not associated with reductions in SV, except in the final interval of LBNP. For variables assessing complexity (i.e., entropy or fractality), there was poor concordance with percentage change in SV across all levels of LBNP. None of the complexity variables showed any consistency beyond two successive LBNP stages.

The analysis of the signs of the change scores is presented in Table 3. Values within the table are the percentage of subjects who had opposite expected signs for any particular HPV metric change compared with the change in sign for SV (i.e., false-negative rate). One minus the table values reflects the sensitivity of the change in the metric to predict percentage change in SV. False-positive values and subsequent specificity were not computed because SV almost always decreased between successive LBNP stages (98% of the time), with the only exception being at the first LBNP stage change. Between 0 and 15 mmHg of LBNP, 72% of the subjects demonstrated an increase in SV. Ignoring the first LBNP pressure change, false-negative rates were consistently low only for SD-1. False-negative rates for RRI root mean squared SD (RMSSD) were moderate (<15%) and fairly consistent.

DISCUSSION

There are numerous data obtained from the clinical literature that have motivated an interest in pursuing the use of HPV as a diagnostic tool to assess the status of trauma patients, particularly those with hemorrhagic injuries (8–11). There are two primary findings in this study that provide new insights into this issue. First, group means for SV and most of the HPV metrics consistently varied with LBNP level. There were similar response functions in both the outcome (SV) and predictor (HPV) variables across progressive LBNP levels. Because of this, evaluation of only the correlation of the means between predictor and outcome variables almost always leads to the conclusion of relationships between LBNP, HPV metrics, and SV. Thus, the high correlations between aggregate mean values at each LBNP level were not unexpected. There is little doubt that, on average, progressive LBNP changes SV as well as a variety of the metrics. Second, none of the changes in HPV metrics as a result of LBNP were consistently correlated with percentage changes in SV. Because the true clinical utility of any particular measurement is only as good as its predictive validity at the individual level, our data support the notion that the HPV metrics investigated herein may be of little usefulness as accurate and reliable means of assessing the degree of central hypovolemia in individuals who are hemorrhaging.

Currently, ECG is readily available in the civilian prehospital trauma setting as it is a standard component of monitoring devices routinely used in the field. Furthermore, future initiatives for monitoring devices in both soldiers and civilian first-care responders contain ECG components that could provide information on injury status to medics not in physical contact with the soldier or first-care responder (29). Indices derived from such a routinely used and accepted monitoring device are therefore attractive because algorithms could be readily embedded into existing monitors and/or future remote monitoring systems. For these reasons, HPV metrics have received a great deal of attention in the literature as potential new "vital signs" for monitoring patients with traumatic injuries. It has been recognized for a number of years that both linear (12, 13, 18) and nonlinear (3, 14, 15, 17, 30) parameters are altered by central hypovolemia induced by either hemorrhage or LBNP. These observations were confirmed in this study and, for the first time, extended to demonstrate strong correlations between the group mean of each examined metric and a direct measurement associated with central hypovolemia (i.e., SV). It is therefore clear from this analysis that, on average, each of these HPV metrics can in fact track central hypovolemia. This initial determination is important because it may reflect the underlying physiological processes associated with compensation to central hypovolemia. Indeed, on average, several of the linear metrics examined are inversely correlated with direct measurements of muscle sympathetic nerve activity during LBNP (20) and may therefore be useful laboratory tools to probe autonomic function when direct microneurography is not possible.

For any metric to be clinically useful for monitoring blood loss, however, it must be able to consistently track the level of hypovolemia across individuals. Despite the concordance of group mean data, none of the metrics provided consistent *individual* tracking of the level of hypovolemia, as clearly illustrated in Figure 2. These data therefore provide compelling evidence that these metrics may be of limited usefulness *on an individual patient basis* to monitor the progression toward development of hemorrhagic shock. Very recently, Tan et al. (31) reached a similar conclusion as to the use of HPV metrics (specifically, entropy and fractality metrics) as diagnostic tools based on their data showing that although group means differed during autonomic perturbation, the metrics failed to distinguish between autonomic disturbances on an individual basis.

Recent evidence from our laboratory further supports this conclusion. First, many of the metrics, particularly those describing the frequency domain (RRI HF and RRI LF), demonstrate an inordinate degree of interindividual variability in resting human subjects (3, 32). Indeed, for every metric (except RRI RMSSD) that demonstrated a direct correlation with SV in this study, there were individual subjects with pre-LBNP baseline values that were less than the group average at the −70 mmHg LBNP level. If group mean values were used

to classify severity of central blood loss, the HPV metrics would have suggested that these individuals had incurred significant central hypovolemia, when in fact they had not been exposed to LBNP. Although we have previously concluded that a single value of any metric is not as useful as monitoring trends over time (3), the current results expand this conclusion by demonstrating very high individual variability in the responses over time to progressive hypovolemia, suggesting that even trends for these particular parameters may not be clinically useful. Porta et al. (33) have also recently demonstrated similar high levels of individual variability in HPV responses to orthostatic stress. In addition, many of these metrics demonstrate poor reproducibility during rest within the same subjects (32), again confirming the difficulty in assigning "normal" ranges and limiting the ability to detect deviations from the healthy condition. Furthermore, some of these metrics are not reproducible during identical autonomic perturbations within the same individuals during different experimental sessions (31). Finally, HPV metrics are invalidated by the presence of ectopic beats and signal noise within the ECG (34–36). Although our data were collected in the controlled environment of a research laboratory, there were data points that had to be eliminated because of the presence of ectopic beats in our population of healthy subjects. We have recently shown that exclusion of ECG records because of the presence of aberrant beats is higher in trauma patients both in prehospital and intensive care unit settings, suggesting that these metrics may not be useful for reliable and continuous monitoring of these patients (37). Taken together, evidence from this and previous work leads to the conclusion that HPV metrics may not be practical for tracking central hypovolemia in trauma patients, particularly as they apply to hemorrhage.

Our study is not without limitations. First, although LBNP provides a suitable test bed for assessment of cardiovascular control during acute central hypovolemia in humans (3, 21–23), it is not accompanied by actual loss of blood or tissue injury. Although some degree of caution must therefore be exercised in applying these conclusions directly to trauma patients, this model provides controlled experimental conditions in which individual responses to central hypovolemia may be assayed without influences that may confound the results (e.g., pain, anxiety, transport conditions, caregiver interventions). Second, rather than being directly measured, SV was noninvasively estimated by computing aortic flow pulsations from arterial pressure waveforms obtained from infrared finger photoplethysmography (25). However, we observed a linear reduction in SV during progressive LBNP similar in relative magnitude to responses previously reported from our laboratory with CO_2 rebreathing (38) and thoracic electrical bioimpedance (39); in fact, in a subset of our 101 subjects, we have previously demonstrated similar decreases of SV during LBNP measured by Finometer and thoracic electrical bioimpedance (24). Thus, although not quantitatively identical in absolute terms, previous data suggest that changes in SV obtained from Finometer arterial pressure waveforms represent a valid estimate of the changes in SV actually occurring during LBNP. Third, the 3-min segments of ECG used at each level of LBNP for determination of HPV metrics contained less than the 800 heartbeats previously recommended for measurement of nonlinear parameters (40). In the analysis reported herein, however, we have used only those nonlinear metrics that we have previously demonstrated remain accurate and valid when 200 heartbeats or fewer are used (28). Fourth, our study was performed using human subjects at rest in the absence of pre-LBNP stressors (e.g., dehydration, anxiety, movement) that might be present in trauma patients; we recognize that sympathoexcitatory stressors might alter baseline levels of HPV metrics (29, 41) but speculate that individual variability in the responses to subsequent LBNP would still occur. Finally, both age and gender may alter both linear and nonlinear metrics (42, 43). In the cohort of subjects studied herein, there were no differences between baseline values of most metrics in males and females; only RRI LF and CDM LF were slightly but significantly lower ($P \leq 0.02$) in females. Furthermore, baseline values were not correlated with age in our subjects ($R^2 \leq 0.20$). Regardless, further study is required to definitively determine the influence, if any, of age and gender on responses of HPV metrics to LBNP.

In conclusion, we demonstrated profound differences in results of statistical analyses based on group means versus those designed to assess individual predictability. Although the group means of each of the HPV metrics accurately tracked changes in SV during central hypovolemia induced by LBNP, the interindividual and intraindividual variability in responses of these metrics precluded reliable determination of the severity of hypovolemia at the individual subject level. Unfortunately, it is not often understood that analyses based on mean change are a necessary but not sufficient condition for clinical validity. This misunderstanding often leads to premature judgments as to the clinical utility of a measurement when applied to decisions made at the patient level. We are not suggesting the abandonment of traditional statistical approaches for the determination of physiological responses during central hypovolemia. Rather, the subsequent step of determining predictive validity at the individual level *must* be performed before advocating any metric for clinical use. Based on the findings of this study, HPV metrics may have limited usefulness for determining the progression of blood loss in individual trauma patients during the early dynamic phase of hemorrhagic injury.

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